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60/474094 U.S. PTO

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV333282775US

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☐ *Additional inventors are being named on the ______ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

COMPOSITIONS AND METHODS FOR THE PREPARATION AND CONJUGATION OF BIANTENNARY POLYMERS INCLUDING POLYETHYLENE GLYCOL

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ENCLOSED APPLICATION PARTS (*check all that apply*)

☑ Specification *Number of Pages* 15 ☐ CD(s), Number ______

☐ Drawing(s) *Number of Sheets* ______ ☑ Other (specify) return postcard

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☑ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502311

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☑ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ______

Respectfully submitted,

SIGNATURE Rachel H. Rondinelli

Date 05/29/2003

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REGISTRATION NO. (*if appropriate*) 45,052

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Docket Number: NEO00262C

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

COMPOSITIONS AND METHODS FOR THE PREPARATION AND CONJUGATION OF BIANTENNARY POLYMERS INCLUDING POLYETHYLENE GLYCOL

This application describes compositions and methods for the preparation and use of biantennary polymers as well as the preparation of mono-dispersed polyethylene glycols (PEGs) and their activated forms. The invention is now described with reference to the following non-limiting Examples and schemes. These Examples and schemes are provided for the purpose of illustration only and the invention should in no way be construed as being limited to these Examples or schemes, but rather should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

I. Compositions and methods for the preparation and conjugation of biantennary polymers

This application describes compositions and methods for the preparation and use of biantennary polymers. The biantennary structure is generated by conjugating the polymer of interest to a small trifunctional ligand in either a step-wise manner or in one pot. Examples of trifunctional ligands that can be used in this invention are described in **Scheme 1**. The more exemplary ligands include epichlorohydrin, 1,3-dibromo-2-propanol, ornithine, glutamate and aspartate. The chemistries of conjugation are well known in the art and include activating such groups as hydroxyl, amine, carboxylate via chemical means to create leaving groups for the subsequent reaction with the polymer. Alternatively, the polymer can be activated and conjugated to the trifunctional ligand.

Exemplary polymers that can be conjugated to create a biantennary structure include PEG (polyethyleneglycol), mPEG (methoxypolyethyleneglycol), mPPG (methoxy-polypropyleneglycol), polysialic acid, polyglutamate, polyaspartate, polylactate and the like. These polymers can be prepared as heterodispersed (polydispersed) or monodispersed forms and used in the conjugation procedures, **Scheme 2.** The heterodispersed mPEG's are prepared by a variety of reported methods with degrees of polymerization ranging from 1 to 20,000 ethylene oxide units. Typically, the mPEGs are separated by size exclusion methodologies and fractionated into ranges of molecular weights. Typically, these ranges are from hundreds to thousands of mass units depending on the size of the PEG. Alternatively, the mPEG is monodispersed, a single molecular weight form, and is prepared by direct chemical syntesis or by separation of a single molecular weight from the polydispersed PEGs.

The activation of the biantennary polymers for conjugation to various ligands can be performed using methods that are standard in the art. For example, a hydroxyl group on the biantennary polymer is

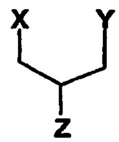
activated with activated forms of carbonate such as the bis-NHS, bis-HOBt or bis-HOAt esters. After activation, the biantennary polymers is conjugated to any suitable ligand such as a protein, nucleotide sugar, peptide, lipid, sugar, DNA, RNA or the like. Exemplary examples are shown in **Scheme 3.**

An example of how to prepare a biantennary PEG is shown in **Scheme 3a.** The method begins with epichlorohydrin and reacts with mPEG under basic reaction conditions. After isolation of the product of the reaction, the biantennary mPEG is activated with the 1,1-bis-HOBt-carbonate to from the biantennary-mPEG-carbonate ester. Reaction of this ester with a nucleotide-sugar then provides a reactant that can be transferred to a protein or glycoprotein by using the appropriate glycosyltransferase.

Additional exemplary embodiments of bi-antennary structures are also described in **Scheme 4** and **Scheme 5.**

Scheme 1. Starting Materials.

a.



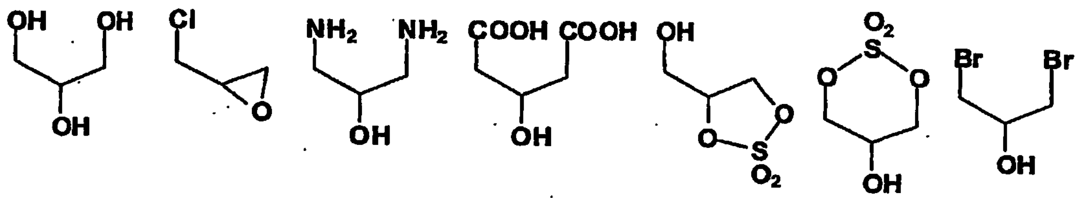
X and Y (independently selected) from OR_1, NR_1R_2, SR_1, alkyl-X, aryl-X, alkylaryl-X, branched alkyl-X, $COOR_1$, $CONR_1R_2$.

Z is selected from OR_1, NR_1R_2, SR_1, $alkylCOOR_1$, $arylCOOR_1$, alkylaryl-X, COOR1, CONR1R2.

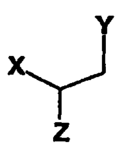
R1 and R2 (independently selected) from H; alkyl; aryl; branched alkyl; R1-R2 as cyclic ring-aromatic, heteroaromatic or alkyl; activating group; halide; leaving group.

Y-Z (independently selected) from a ring such as epoxide, aziridine, cylic-sulfonate.

Exemplary examples:



b.



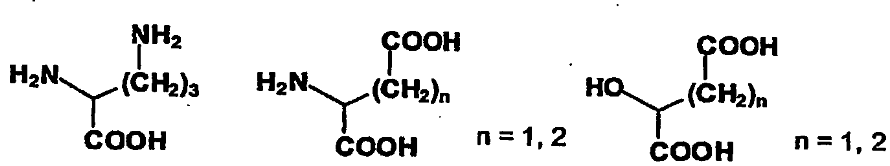
X and Y (independently selected) from OR_1, NR_1R_2, SR_1, alkyl-X, aryl-X, alkylaryl-X, branched alkyl-X, $COOR_1$, $CONR_1R_2$, except when X is NH_2 and Y is $(CH_2)_3NH_2$.

Z is selected from OR_1, NR_1R_2, SR_1, $alkylCOOR_1$, $arylCOOR_1$, alkylaryl-X, $COOR_1$, $CONR_1R_2$.

R_1 and R_2 (independently selected) from H; alkyl; aryl; branched alkyl; R_1-R_2 as cyclic ring-aromatic, heteroaromatic or alkyl; activating group; halide; leaving group.

Y-Z (independently selected) from a ring such as epoxide, aziridine, cylic-sulfonate.

Exemplary examples:



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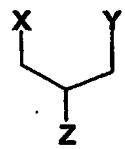
Scheme 2. Branched PEG's.

a.



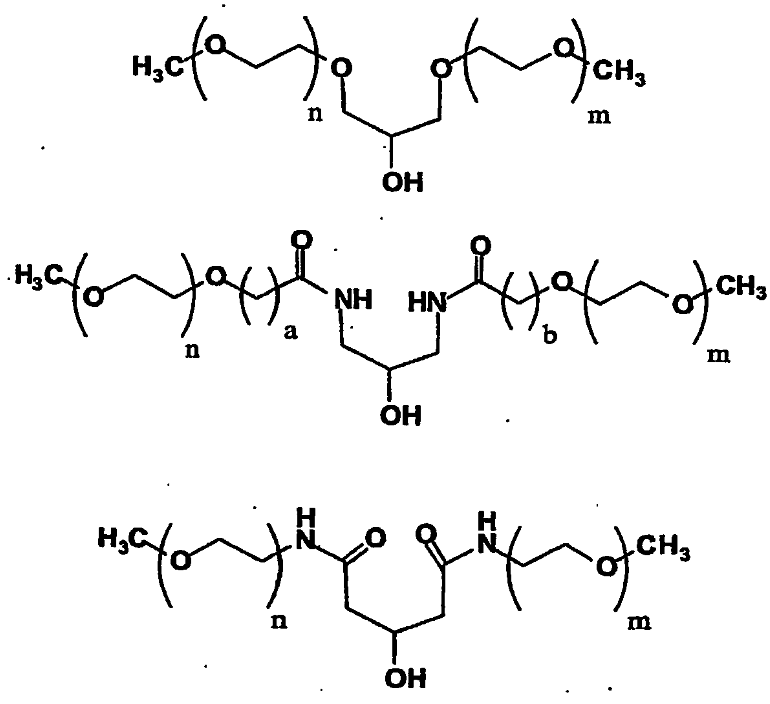
X and Y (independently selected) from OR_1, NR_1R_2, SR_1, alkyl-X, aryl-X, alkylaryl-X, branched alkyl-X, $COOR_1$, $CONR_1R_2$.

Z is selected from OR_1, NR_1R_2, SR_1, alkyl$COOR_1$, aryl$COOR_1$, alkylaryl-X, $COOR_1$, $CONR_1R_2$.

R_1 and R_2 (independently selected) from H; alkyl; aryl; branched alkyl; R_1-R_2 as cyclic ring-aromatic, heteroaromatic or alkyl; activating group; mPEG, PEG, mPPG, polysialic acid, polyglutamate, polyaspartate, polylysine, polyethyleneimine, polylactide, polyglyceride, functionalized PEG, polymer.

PEG is polyethyleneglycol; mPEG is methoxypolyethyleneglycol; mPPG is methoxypolypropyleneglycol;

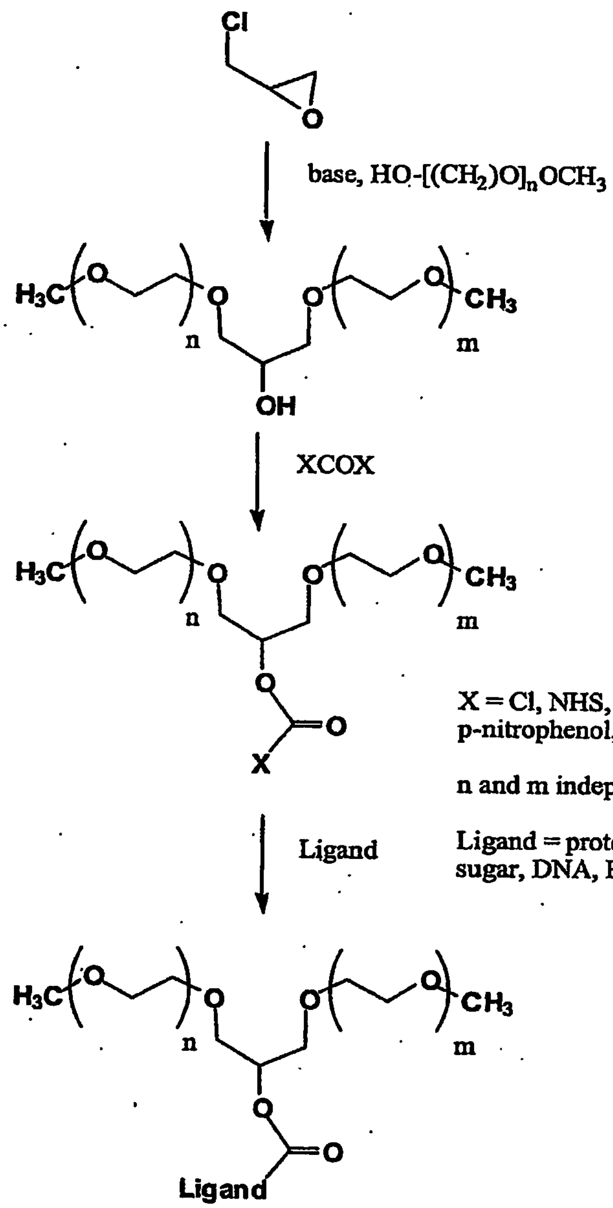
Exemplary examples:



a, b, n and m (independently selected) from 1 to 20,000.

Scheme 3. Activated and Coupled Biantennary Polymers.

a. Exemplary Examples.



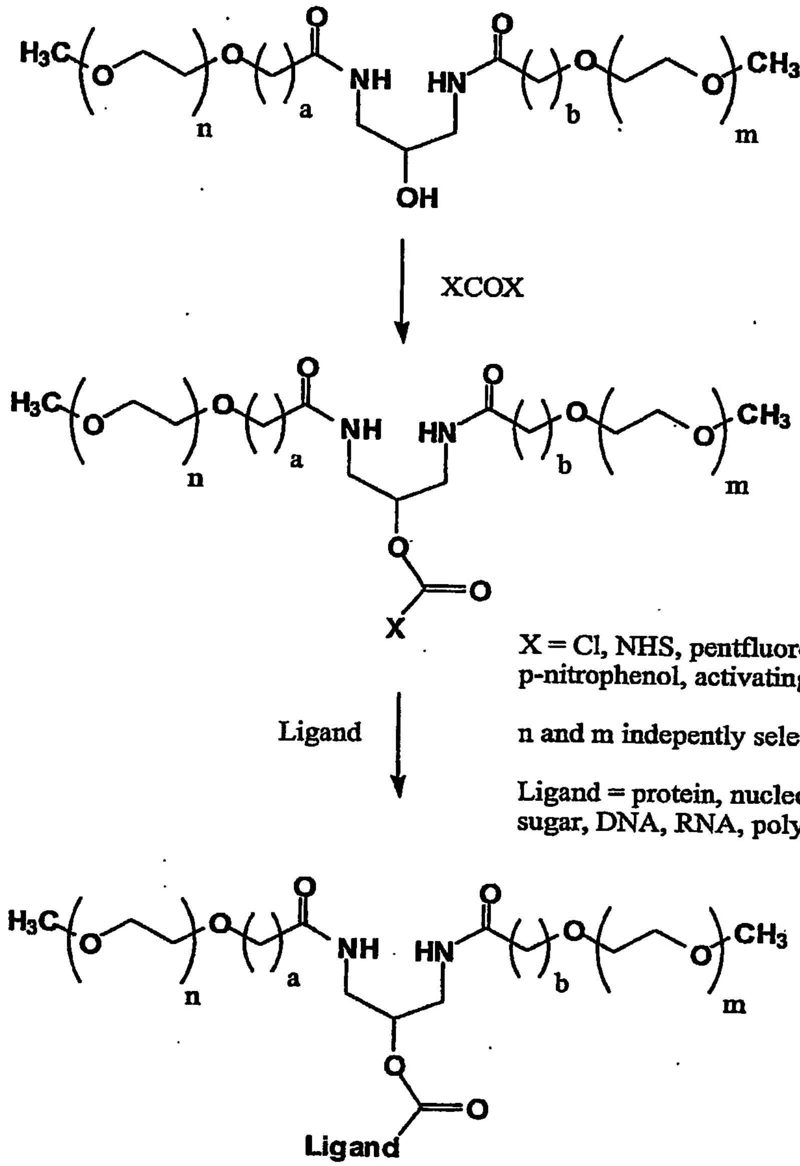
X = Cl, NHS, pentfluorophenol, HOBT, HOAt, p-nitrophenol, activating group.

n and m indepently selected from 1 to 20,000.

Ligand = protein, nucleotide sugar, peptide, lipid, sugar, DNA, RNA, polymer, peptide.

Scheme 3. Activated and Coupled Biantennary Polymers.

b. Exemplary Examples.



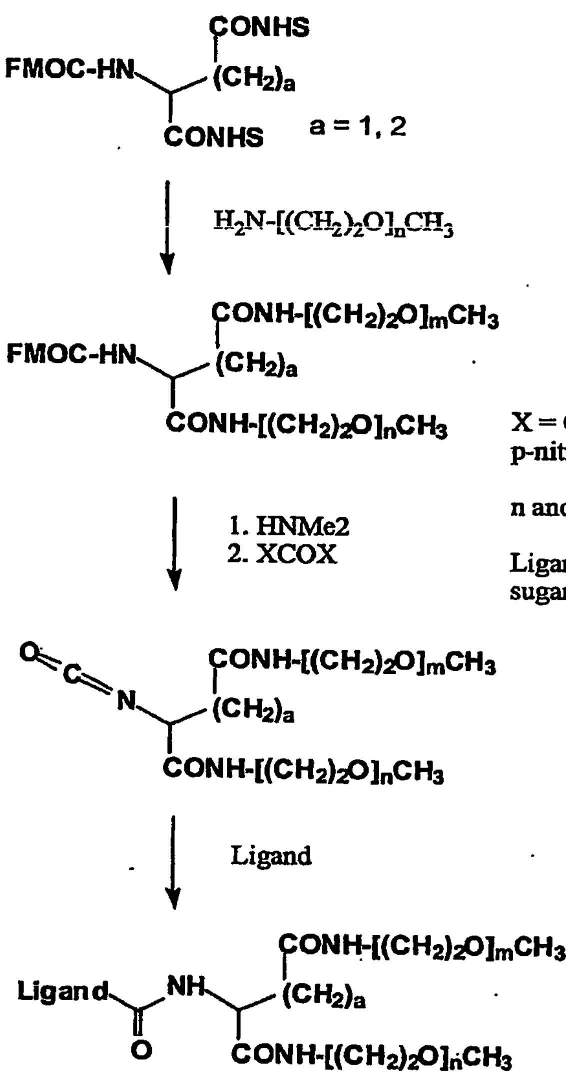
X = Cl, NHS, pentfluorophenol, HOBT, HOAt, p-nitrophenol, activating group.

n and m indepently selected from 1 to 20,000.

Ligand = protein, nucleotide sugar, peptide, lipid, sugar, DNA, RNA, polymer, peptide.

Scheme 3. Activated and Coupled Biantennary Polymers.

c. Exemplary Example.

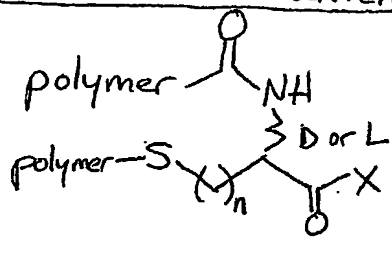


X = Cl, NHS, pentfluorophenol, HOBT, HOAt, p-nitrophenol, activating group.

n and m indepently selected from 1 to 20,000.

Ligand = protein, nucleotide sugar, peptide, lipid, sugar, DNA, RNA, polymer, peptide.

Scheme 4. Additional Bi-antennary structures.

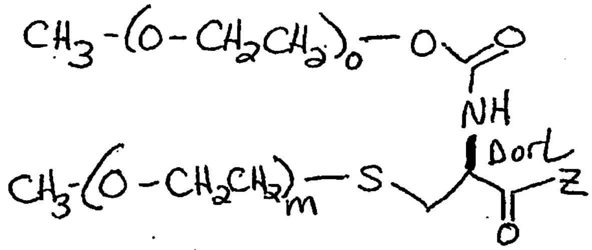


; wherein X = OH, H, an activating group, or a nucleotide sugar;

and n = 1-10;

and polymer = PEG, PPG, mPEG, mPPG, alkyl-PEG, polyglutamate, polysialic acid, and the like.

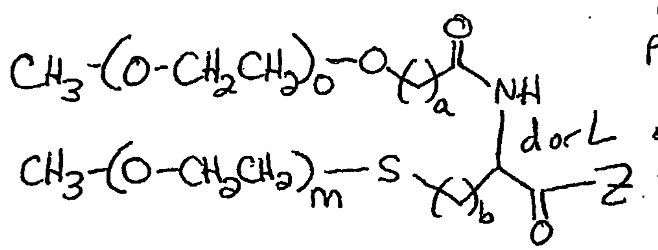
Specific examples include, but are not limited to:

a) 

wherein O = 1-10,000;

m = 1-10,000;

and Z = OH, H, an activat group such as HOBT, HOAT, N-hydroxysuccinimide ester, p-nitrophenol ester, and the li

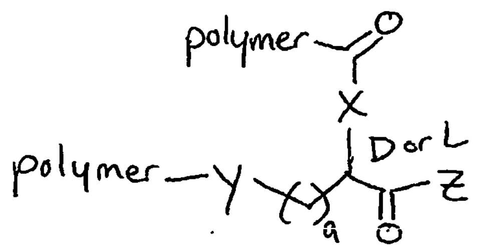
b) 

; Wherein m, O = independ selected from 1-29

a,b = independently selected from 1-24

and Z = OH, H, an activating group such as HOBT, HOAT, N-hydroxy-succinimide ester, p-nitrophenol ester, and the like.

Scheme 5. Additional Bi-antennary Polymers



wherein X, Y = independently selected from O, N, S;

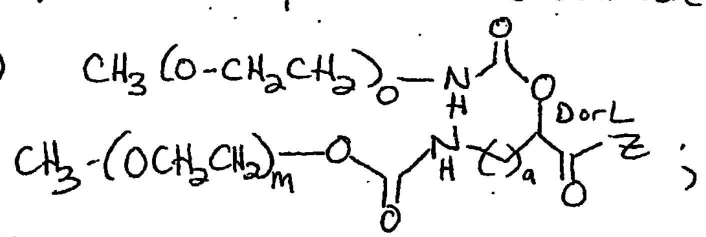
a = 1-10;

Z = OH, H, an activating group, a nucleotide sugar

and polymer = PEG, PPG, mPEG, mPPG, alkyl-PEG, polyglutamic acid, polysialic acid, and the like.

Specific examples include but are not limited to:

a)

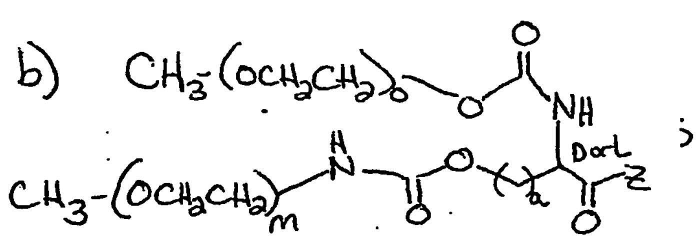


wherein m, o = independently selected from 1-20,000;

a = 1-10; and

Z = OH, H, an activating group such as HOBT, HOAT, N-hydroxysuccinimide ester, p-nitrophenol ester, and the like.

b)



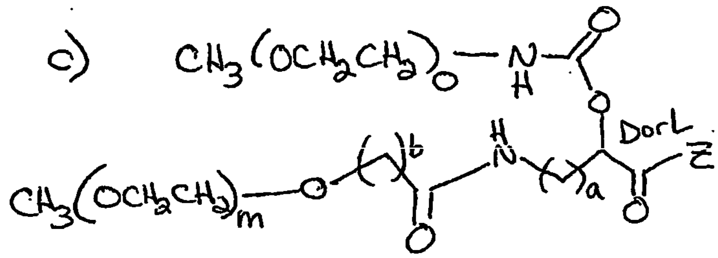
wherein m, o = independently selected from 1-20,000;

a = 1-10; and

Z = OH, H, an activating group such as HOBT, HOAT, N-hydroxysuccinimide ester, p-nitrophenol ester, and the like

Scheme 5 continued. Additional Bi-antennary Polymers

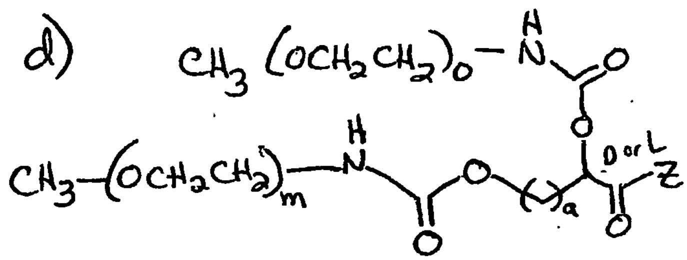
c)



; wherein m, o = independently selected from 1-20,000; a = 1-10; b = 1-24; and Z = OH, H, an activating group such as HOBT, HOAT, N-hydroxysuccinin ester, p-nitrophenol ester, and the like.

and

d)

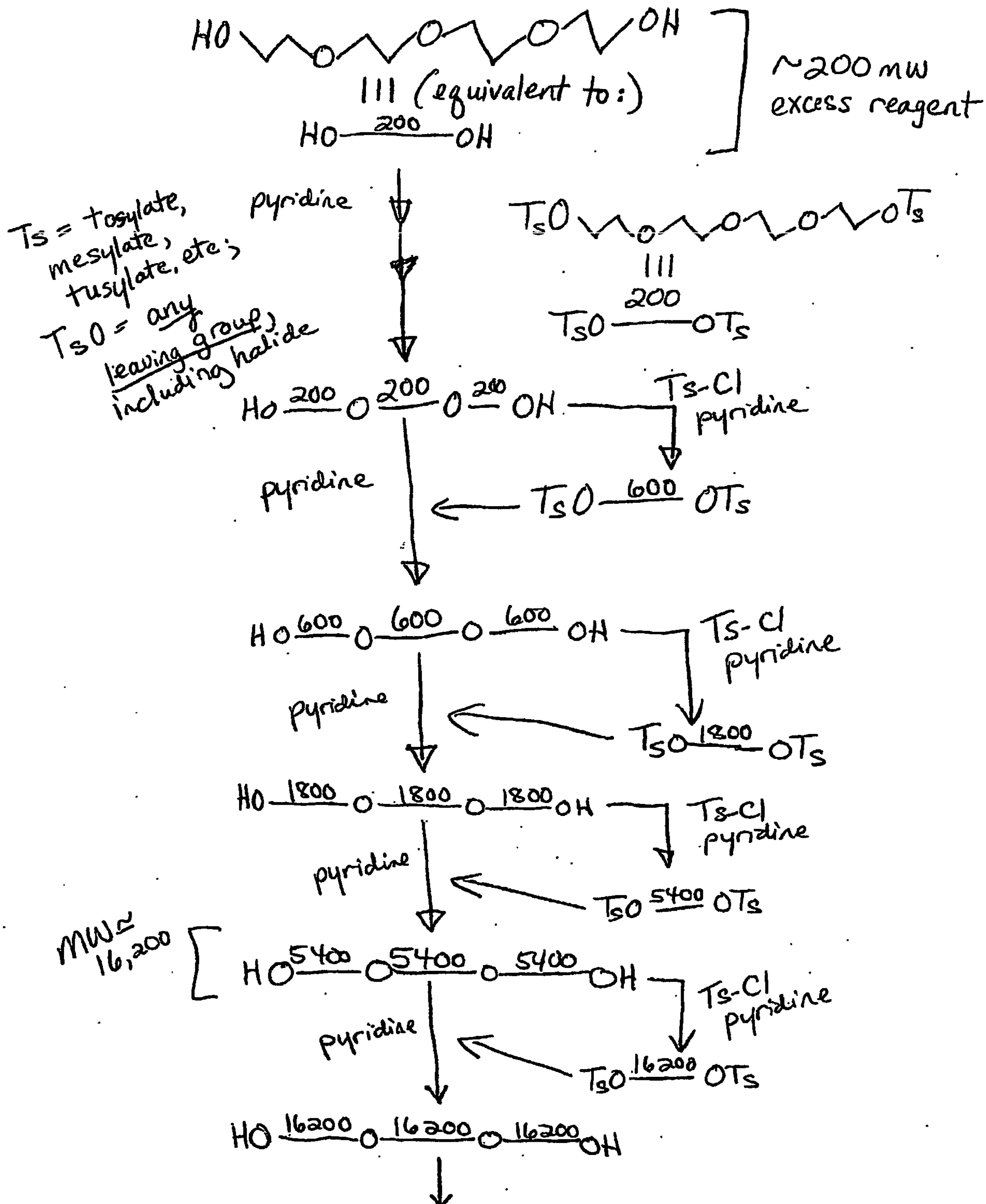


; wherein m, o = independently selected from 1-20,000; a = 1-10; and Z = OH, H, an activating group such as HOBT, HOAT, N-hydroxysuccin ester, p-Nitrophenol ester, and the like.

II. Preparation of mono-dispersed polyethylene glycols (PEGs) and their activated forms

This application also describes preparation of mono-dispersed polyethylene glycols (PEGs) and their activated forms. Mono-dispersed or singular molecular weight PEGs can be prepared as shown below. By adjusting the size of the fragments generated, any size PEG can be prepared. The diols can then be converted to their mono-methoxy derivatives and then activated for conjugation to protein sugar, lipid, nucleotide sugars or DNA/RNA.

Example 1:



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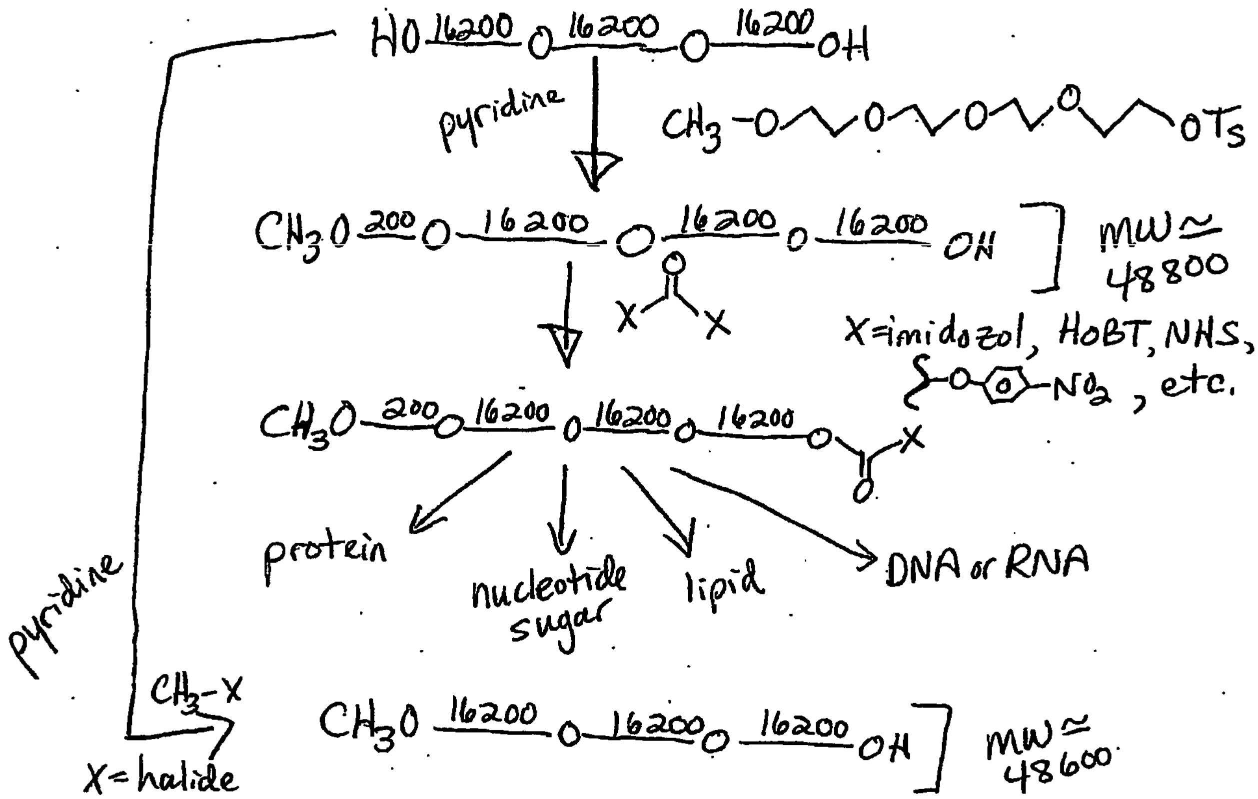
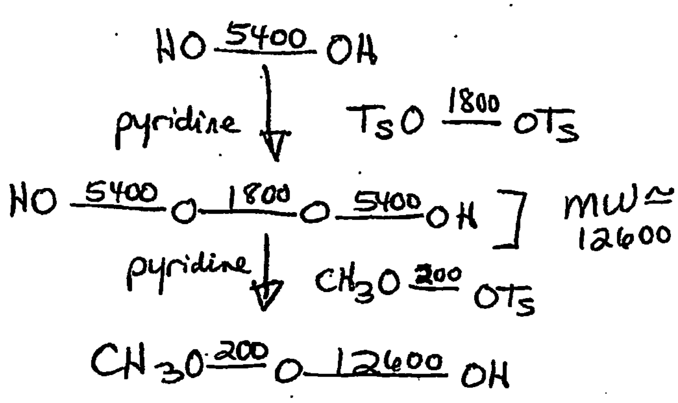
Example1: continued



Example 2:



Example 3:

$$HO \overset{200}{\text{——}} OH$$

$$T_sO \overset{200}{\text{——}} OT_s \quad (\text{excess})$$

$$\downarrow$$

$$HO-(O-O)_n-OH \quad ; \text{ wherein } n = 1\text{-}20{,}000$$

By varying the ratio of reactants, the base used, temperature, solvent and concentration, one can adjust the reaction to give the predominant size (n) desired.

This approach describes a simple, fast, efficient way to prepare the polyethylene glycols, of any size, in a mono-dispersed size. Purification is simplified by this approach because the difference in size and therefore each molecule's physico-chemical characteristics is very different. This allows the use of simple, standard purification techniques such as silica gel, reversed phase, cellulose, membrane filtration (nano-filtration and ultra-filtration) to be used. The purified PEG diols can then be derivatized into any functional form that is desired.

While this invention has been disclosed with reference to specific embodiments, it is apparent that other embodiments and variations of this invention may be devised by others skilled in the art without departing from the true spirit and scope of the invention.